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Art. XV.—*On* Saprolegnia terrestris *sp. nov., with some Preliminary Observations on Victorian Soil Saprolegniales.*

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Amongst the several Phycomycetes that have been isolated from soil samples taken from the fern gullies of the Dandenong Ranges near Melbourne, a characteristic form of *Saprolegnia* has been present. This form has been cultured for some considerable period on hemp seed pieces in sterilized tap water and has also been isolated several times from fresh soil samples. Throughout it has maintained certain constant characteristics which appear to distinguish it from any previously described species. The fact that considerable variations in both habit and form occur in cultures of the so-called species of *Saprolegnia* and *Achlya* has been recognized and considered. Nevertheless the conclusion reached after careful observation is that this form which has been isolated, and is to be described below, is an hitherto unrecorded species of *Saprolegnia*. It will be known as *Saprolegnia terrestris*.

S. terrestris grows well on hemp seed pieces in sterilzed tap water, and in some such cultures fertile oogonia develop abundantly without special treatment. Frequently, however, it has been necessary to induce the formation of oogonia by the method suggested by Couch (1932). In such cases young cultures on hemp seed were grown for three days in a 2 per cent. solution of peptone; they were then washed in tap water, and subsequently grown in sterilized tap water for about seven days. After such treatment large numbers of oogonia were usually produced. The following diagnosis has been made from hemp seed cultures with or without growth for a period in 2 per cent. peptone solution.

Diagnosis.—Growth on hemp seed about 0.5–1 cm. long, mycelial mat thick. Hyphae slender, up to 48μ broad at the base. Sporangia abundant, very variable in shape, typically cylindrical or clavate 16–48μ broad, 60–400μ long, frequently almost spherical sometimes irregular and contorted, opening apically by a more or less prominent mouth. Primary sporangia terminal; secondary sporangia develop either by internal proliferation or by the delimitation of a segment behind a discharged sporangium and the outgrowth from this segment of a sporangium; occasionally secondary sporangia develop in a truly cymose manner. Spores diplanetic about 10.5μ when encysted. Gemmae usually not abundant, cylindrical, pyriform, or irregular in shape. Oogonia borne terminally or laterally on straight stalks which are as long as, or considerably longer than, the diameter of the

FIG. 1.—*Saprolegnia terrestris* sp. nov. Types of sporangia. × 125. Gemmae. × 125.

oogonium, sometimes intercalary; typically spherical, sometimes with a neck, or, when developed within an empty sporangium, cylindrical, occasionally with a short apiculus, 30–87.5μ in diameter, average 61μ. Oogonial wall usually yellow, frequently unpitted but in some cultures with well defined though not conspicuous pits. An upgrowth from the basal wall of the oogonium is frequently present, and an irregular internal thickening of the oogonial wall is sometimes met with in old hemp seed cultures. Oospores 1–10 usually 2–6, 20–37μ in diameter, average 29μ, dark brown when immature, later becoming yellowish; eccentric in sense that the peripheral sheath of oil drops does not completely surround the protoplasm, or subcentric. Antheridia present on all oogonia, typically one, sometimes two or three; antheridial branches androgynous, usually simple, but sometimes slightly branched, typically arising from the oogonial stalk immediately behind the oogonium but occasionally developing from the same hypha as the oogonium; antheridia clavate attached by their sides to the oogonium, becoming inconspicuous; antheridial tubes large and conspicuous.

In considering the affinities of *S. terrestris,* the structure of the oospore is of considerable importance. This, in *S. terrestris,* has an unusual form and one that is almost unique for the genus *Saprolegnia.* In a large number of the mature spores the spherical protoplasmic body is incompletely surrounded by fat

droplets, these being entirely lacking on part of one side (Fig. 2, D, G, H, and Plate XI., figs. 6, 9, 10, 15). Eggs with a similar construction occur, according to Coker (1923), in *Aehlya oblongata* de Bary, and in *Aplanes Treleaseanus* (Humphrey) Coker (1927). In both cases they have been described as subcentric. I propose to distinguish, by the use of the term "eccentric," the eggs of *S. terrestris* from the typical subcentric type in which the protoplasm is surrounded by one layer of oil drops on one side and two or three on the other. Frequently

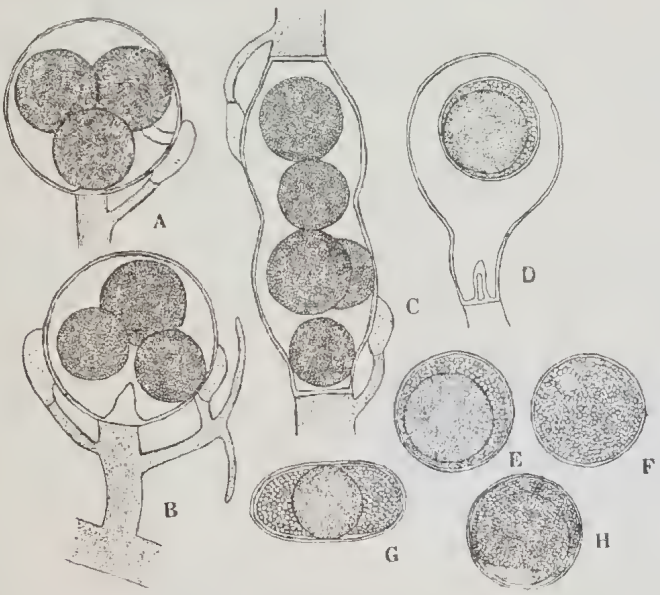

FIG. 2.—*Saprolegnia terrestris* sp. nov. (*a*) A spherical oogonium with antheridium showing fertilization tube. × 285; (*b*) Oogonium showing basal ingrowth. × 285; (*c*) An intercalary oogonium. × 285; (*d*) An oogonium containing a single eccentric oospore. × 440; (*e*) An apparently subcentric oospore in optical section. × 440; (*f*) The same oospore focussed to show that the peripheral sheath of oil drops is incomplete. × 440; (*g*) An elliptical oospore with two lunate masses of oil drops. × 440; (*h*) an eccentric oospore. × 440.

eggs of *S. terrestris* when viewed in optical section appear to be subcentric (Fig. 2, E). However, when the focus is changed a small clear area from which oil drops are absent will usually be seen at some point in the peripheral sheath (plate XI., Fig. 7; Fig. 2, F). In these eggs also the protoplasm is incompletely invested. In addition a small number of truly subcentric eggs occur in most cultures. It will be seen therefore that the oospores of *S. terrestris* range from the eccentric to the subcentric condition.

The only species of *Saprolegnia* in which the oospores are said to be eccentric is the plant isolated and described by Von Minden (1912), and referred by him to Cornu's inadequately described species *S. spiralis* (1872). In Coker's translation of

Von Minden's description on page 71 of "The Saprolegniaceae," the eggs are described as follows:—"Eggs mostly 1–2 seldom 3 or even 4, smooth, spherical or when several more or less elongated, at maturity with one or many mostly lateral fat drops and therefore more or less plainly eccentric." As no figures are given, it is impossible to say whether or not their structure agrees exactly with that of the eggs of *S. terrestris*, but Von Minden's statement that the eggs of his plant are "more or less plainly eccentric" necessitates a comparison of the two forms.

Apart from the spore characteristics previously mentioned, there are several morphological features in which the two plants agree exactly. The most important are the mode of development of the secondary sporangia, either by proliferation or "cymose" development; the position of the oogonia, either lateral, terminal, or intercalary; the occasional presence of a blunt extension of the tip of the oogonium, the inconspicuous pitting, or entire absence of pits from the oogonial walls; the size of the oogonia and the approximation in size of the oospores; the androgynous antheridial branches, which develop either from the stalk of the oogonium or from the main hyphae and which become inconspicuous.

Several differences, however, are evident. The secondary sporangia are always abundantly developed in *S. terrestris*, whereas they are reported to be few in number in *S. spiralis*. More important is the fact that the oogonial stalks are not bent or spirally coiled as in *S. spiralis*. Further, the oogonia of the latter are usually "elliptic or ovate," whereas in *S. terrestris* they are typically spherical. There is no mention in Von Minden's description of the colouration of the oogonial walls or of the in-growth from the basal wall of the oogonium, both of which are usual features of *S. terrestris*. The antheridial branches are simpler in the latter, and are never "numerous and much branched" as in *S. spiralis*; further, they only rarely develop away from the oogonial stalk in *S. terrestris*. The number of eggs is on the average higher in the latter, being usually 2–4, and as high as 10, and the eggs are slightly larger, with an average of 29μ as against an average of 26μ in *S. spiralis*. It is because these differences exist that I have decided to regard the two plants as specifically distinct. I have also been influenced in making this decision by the rather questionable systematic position of Von Minden's plant, and by the doubt that exists, in the absence of figures, as to the detailed structure of its oospores.

Of the other described species of *Saprolegnia*, those with subcentric spores, namely, *S. megasperma* Coker and *S. asterophora* de Bary are of interest. Both, however, are quite distinct from *S. terrestris* in several important characters. The possibility that *S. retorta* Horn (1904) is synonymous with *S. spiralis* Cornu as suggested by Von Minden is not important in this discussion since *S. terrestris* differs from Horn's plant in such constant

features as the size and structure of the oospores, the absence of spirally coiled oogonial stalks as well as in the copious production of sporangia.

Amongst the centric spored species of *Saprolegnia* the one to which *S. terrestris* approaches most nearly is *S. littoralis* Coker. The characters in which the two forms agree are those which they share in common with Von Minden's plant described as *S. spiralis* Cornu which Coker thinks may possibly be identical with *S. littoralis*. *S. terrestris,* however, is distinct from S. *littoralis* in the less abundant and simpler antheridial branches, in the presence of an in-growth from the basal wall of the oogonium as well as in the structure of the oospores.

Some Preliminary Observations on Victorian Soil Saprolegniales.

The presence in soil of the so-called aquatic fungi was demonstrated by Harvey in 1925. Since then the work of Coker (1926, 1927), Couch (1927), and Harvey (1930) in America, of Nagai (1931) in Japan, and of Cook and Morgan (1934) in Wales has shown that many members of Saprolegniales are normal components of the soil flora. With a view to ascertaining the particular forms that are present in Australian soils some preliminary isolations from three distinct types of soil have been made. The investigation, which, it is proposed, shall include a study of the ecological distribution and seasonal occurrence of the Saprolegniaceous forms, is as yet in its preliminary stages. The number of isolations that have been made is comparatively small and the list of species given below is consequently incomplete. No conclusions as to the distribution of the individual species can be drawn from it.

The method adopted for the isolation from soil samples has been that suggested by Harvey (1925). Soil was taken to a depth of 3 inches and placed in crystallizing dishes. It was covered with sterilized tap water and when the water had cleared, boiled pieces of hemp seed were sown on the surface of the soil. Pure cultures on either corn-meal agar or malt agar were obtained from the fungal growths that appeared on the hemp seed and from the agar cultures pure hemp seed colonies were secured of the individual types isolated. The specified identification has been based throughout on such pure hemp seed cultures.

In this preliminary work soil has been taken from three localities:—

Locality A.—Bush soil of a clay character which supports an endemic flora, at Ringwood near Melbourne. Rainfall 30 inches but subject to severe periods of drought during the summer months.

Locality B.—Rich humus soil from fern gullies in the Dandenong Ranges near Melbourne. Rainfall approximately 40 inches. Soil moist all the year round except perhaps in unusually dry seasons.

Locality C.—Cultivated soil from the System Garden of the University of Melbourne. Rainfall 26 inches. Artifically watered during the summer.

Locality A.—Ringwood.

ACHLYA cf. IMPERFECTA Coker in "The Saprolegniaceae" 1923.

A species of *Achlya* has been isolated which appears to be nearer to *A. imperfecta* Coker than to any other species of *Achlya*. The chief variation from the type is in the size of the oospores, which are considerably larger in the Victorian plant.

Diagnosis.—Sporangia and spores as in the species. Oogonia spherical, occasionally with an apiculus, 50–90μ in diameter, racemosely borne on long or short stalks; oogonial wall smooth and frequently unpitted, sometimes showing several inconspicuous pits, in-growth from the basal wall frequently present. Oospores eccentric with a single large oil drop, 1–10 in an oogonium, 22.5–32.5μ in diameter, average 26.5μ many degenerating. Antheridial branches androgynous and diclinous, but more frequently androgynous, sometimes arising from the oogonial stalk, frequently much branched and occasionally almost completely investing the oogonium.

Lund (1934) has identified a plant from Danish soil as *A. imperfecta* Coker which differs from the type in the greater size of the oospores, 22.5–27.5μ as against 17–23μ, and in the more numerous pits in the oogonial wall. The Victorian plant approaches Lund's form in the size of the eggs, but differs from it and agrees with the type in the infrequent pitting of the oogonial wall. It is felt that further observations are necessary before a more definite and satisfactory statement as to the identification of the Victorian species can be made.

ACHLYA APICULATA de Bary, Bot. Zeit. xlvi., p. 635, 1888.

In general agreement with the type. The oogonial apiculus was seldom seen. In old hemp seed cultures the irregular internal thickening of the oogonial wall mentioned by Coker and Couch in their descriptions of *A. apiculata* var. *prolifica* was observed. Oogonia 45–125μ in diameter. Oospores 32–47.5μ.

Locality B.—Dandenong Ranges.

SAPROLEGNIA TERRESTRIS sp.nov., described above.

From fern gullies at Upper Ferntree Gully, Kallista, and Kalorama.

Saprolegnia magasperma Coker, "The Saprolegniaceae," 1923.

Similar to the type. Oogonia 35–70μ. Oospores 27–50μ, single in 90 per cent. of the oogonia. From Upper Ferntree Gully. This species has not been previously recorded from the soil.

Achlya racemosa Hilderbrand, Jahrb. f. wiss. Bot. 6, p. 249, 1867.

Identical with the type. Oospores 22.5–27.5μ. From Kallista.

Achlya apiculata de Bary.

Similar to cultures obtained from Locality A.

Achlya cf. imperfecta as from Locality A.

Thraustotheca clavata (de Bary) Humphrey, Trans. Am. Phil. Soc. xvii., p. 131, 1892 (1893).

In exact agreement with the type.

Locality C.—University Grounds.

Saprolegnia anisospora de Bary, Bot. Zeit. xlvi., p. 619, 1888.

General characters are in agreement with the type. Large zoospores ranged in diameter from 15–20μ, the small ones were 10μ. In all cultures the large zoospores greatly exceeded the small ones in numbers. This was not the case in Coker's material. Contrary also to the experience of both Coker (1923) and Nagai (1931), the oogonia were difficult to obtain. Small oogonia developed when young hemp seed cultures were grown in 2 per cent. peptone solution, and subsequently in sterilized tap water. Both oogonia and oospores were slightly smaller than is usual, the oogonia ranging from 25–45μ and the oospores from 11–25μ, but this may have been due to the means by which their formation was induced. Not previously recorded from soil.

Saprolegnia terrestris n.sp.

In exact agreement with material from Locality B.

Achlya caroliniana Coker, Bot. Gazette, l., p. 381, 1910, and Coker and Braxton, J. Elisha Mitchell Sci. Soc., vol. xlii., p. 139, 1926.

This species was obtained from each isolation made, characters agree with those of type. Oogonia 20–50μ. Oospores 14–25μ, up to 6 per oogonium. Antheridial branches diclinous or androgynous disappearing with age of the culture.

Isoachlya unispora Coker and Couch in "The Saprolegniaceae," 1923.

Characters in agreement with the type with the exception that sporangia were abundant in all cultures and were developed either within or without the empty sporangium not entirely without it as in Coker's material. Oogonia 27.5–45μ. Oospores 22.5–42.5μ.

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Explanation of Plate.

All figures are of *Saprolegnia terrestris* n. sp. Photographs were taken of living material in water and prints were made from unretouched negatives.

Figs. 1-4. Show the mode of formation and variation in shape of sporangia. × 120.

Fig. 5. An oogonium showing typical origin of antheridial branch and the presence of a pit in the oogonial wall. × 160.

Fig. 6. An oogonium showing two eccentric oospores. × 490.

Fig. 7. Two oospores of a cylindrical oogonium; the left hand egg shows subcentric structure, the right hand one is focussed to show the incompleteness of the peripheral sheath of oil drops. × 490.

Fig. 8. A typical oogonium and antheridium. × 120.

Fig. 9. An oogonium with a neck, a terminal apiculus and one eccentric oospore. × 360.

Fig. 10. A spherical oogonium with two elliptical oospores in which the oil drops are restricted to two lunate areas. × 490.

Fig. 11. A cylindrical oogonium within an empty sporangium, showing an antheridial branch and an upgrowth from the basal wall. × 490.

Fig. 12. Hypha showing lateral origin of oogonia on long, straight stalks. × 40.

Fig. 13. A young oogonium and antheridium. × 120.

Fig. 14. One of the rare instances in which the antheridial branch has arisen from the hypha bearing the oogonium. × 160.

Fig. 15. An eccentric spore. × 720.

Saprolegnia terrestris n. sp.